(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2003 (18.09.2003)

PCT

(10) International Publication Number
WO 2003/076603 A3

(51) International Patent Classification[7]: C12N 5/08, A61K 39/12

(21) International Application Number: PCT/IB2003/001391

(22) International Filing Date: 13 March 2003 (13.03.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/363,849 14 March 2002 (14.03.2002) US

(71) Applicant *(for all designated States except US)*: ANOSYS, INC. [US/US]; 1014 Hamilton Court, Menlo Park, CA 94025 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HOU, Yafei [CN/US]; 2050 Latham Street, Apt #36, Mountain View, CA 94040 (US). HSU, Di-Hwei [US/US]; 1030 El Camino Real, Apt #168, Sunnyvale, CA 94087 (US). MEHTA-DAMANI, Anita [US/US]; 570 Connemara Way, Sunnyvale, CA 94087 (US). LAMPARSKI, Henry [CA/US]; 141 41st Avenue, San Mateo, CA 94403 (US). PAZ, Pedro [US/US]; 133 Rocky Point Court, Hercules, CA 94547 (US). LE PECQ, Jean-Bernard [FR/US]; 675 Olive Street, Menlo Park, CA 94025 (US).

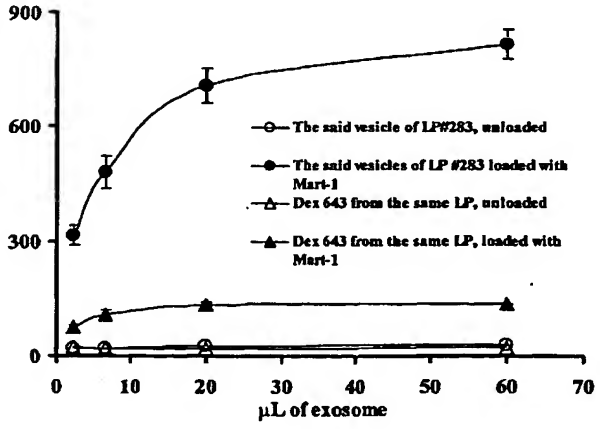
(74) Agents: BECKER, Philippe et al.; Becker et Associés, 35, rue des Mathurins, f-75008 Paris (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: FUNCTIONALIZATION OF T CELL DERIVED VESICLES AND USE THEREOF FOR THE PREPARATION OF IMMUNOGENIC PHARMACEUTICAL COMPOSITIONS



(57) Abstract: The present invention relates to compositions comprising vesicles released' from activated T lymphocytes, as well as to methods for their production and uses. Said vesicles contain a set of bioactive molecules which confer remarkable properties, such as antigen recognition, antigen presentation and other regulatory and effector functions. This invention also relates to methods for transferring or delivering antigenic molecules (e.g., peptides, peptide/MHC complexes, TCR or subunit thereof, etc.) to antigen presenting cells (APCs) using said vesicles, to induce specific immune responses, particularly specific CTL responses. The invention further relates to methods of delivering molecules selectively or specifically to target cells using said vesicles.

WO 2003/076603 A3

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
22 January 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Intern I Application No
PCT/IB 03/01391

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 A61K39/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARTINEZ-LORENZO, M.J. ET AL.: "Activated Human T Cells Release Bioactive Fas Ligand and APO2 Ligand in Microvesicles" JOURNAL OF IMMUNOLOGY, vol. 163, no. 3, 1 August 1999 (1999-08-01), pages 1274-1281, XP002250995 the whole document see especially: abstract page 1277, column 2, line 21 - page 1279, column 1, line 44 and: page 1279, column 2, line 5 - page 1280, column 1, line 20	1-10, 12-15, 22-28, 30,31

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2003	18. 11. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr I Application No
PCT/IB 03/01391

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01 82958 A (AP CELLS, INC.) 8 November 2001 (2001-11-08) cited in the application abstract page 2, line 30 - page 14, line 10 page 26, line 12 - line 16 page 46 - page 61; examples 1-12 page 62 - page 69; claims 1,4-8,10-18,28,29,32-46,55-65,79 -----	1-10, 12-15, 22-28, 30,31
A	DENZER, K. ET AL.: "Exosome: from internal vesicle of the multivesicular body to intercellular signaling device" JOURNAL OF CELL SCIENCE, vol. 113, no. 19, October 2000 (2000-10), pages 3365-3374, XP002225014 cited in the application abstract page 3366, column 2, line 27 - line 43 page 3367, column 2, line 26 - column 1, line 26 page 3372, column 1, line 9 - column 2, line 7 -----	1-10, 12-15, 22-28, 30,31

INTERNATIONAL SEARCH REPORT

Ir tional application No.
PCT/IB 03/01391

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 24, 25, 27 and 28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9,12-15,22-28,30,31 (all partially) and 10 (completely)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-9, 12-15, 22-28, 30, 31 (all partially) and 10 (completely)

a method of producing lipid vesicles comprising: a) culturing a biological preparation comprising T lymphocytes, and b) collecting or purifying vesicles produced in a) wherein said vesicles are functionalized to express a selected molecule by direct loading, a method of producing immunogenic vesicles comprising: a) culturing a biological preparation comprising T lymphocytes, b) collecting or purifying vesicles produced in a), and c) contacting said vesicles with an antigenic molecule under conditions allowing the molecule to bind said vesicles, said method wherein the antigenic molecule comprises a HCV envelope glycoprotein or a CD81-binding fragment thereof, a pharmaceutical composition comprising a directly loaded T cell derived membrane vesicle and a pharmaceutically acceptable vehicle or excipient, a method of stimulating an immune response against an antigen comprising administering an effective amount of said composition, a method of delivering an antigenic molecule to an antigen-presenting cell involving said composition, a method of stimulating dendritic cells involving said composition, a method of delivering a molecule to a target cell involving said composition, a composition comprising an immunogenic T cell derived membrane vesicle directly loaded with an antigenic molecule, a composition comprising a vesicle derived from T lymphocyte and a HCV envelope glycoprotein or a CD81-binding fragment thereof;

---

2. claims: 1-9, 12-15, 22-28, 30, 31 (all partially) and 11, 16 (all completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a method of producing lipid vesicles comprising: a) culturing a biological preparation comprising T lymphocytes, and b) collecting or purifying vesicles produced in a) wherein said vesicles are functionalized to express a selected molecule by chimeric loading, a method of producing immunogenic vesicles comprising: a) culturing a biological preparation comprising T lymphocytes, b) collecting or purifying vesicles produced in a), and c) contacting said vesicles with an antigenic molecule under conditions allowing the molecule to bind said vesicles, said method wherein the antigenic molecule comprises a HCV envelope glycoprotein or a CD81-binding fragment thereof, said method wherein the molecule is a chimeric protein comprising a polypeptide fused to lactadherin or to HCV glycoprotein, a pharmaceutical composition comprising a chimericaly loaded T cell derived membrane vesicle and a pharmaceutically acceptable vehicle or excipient, a method of stimulating an immune response against an antigen comprising administering an effective amount of said composition, a method of delivering an antigenic molecule to an antigen-presenting cell involving said composition, a method of stimulating dendritic cells involving said composition, a method of delivering a molecule to a target cell involving said composition, a composition comprising an immunogenic T cell derived membrane vesicle chimericaly loaded with an antigenic molecule, a composition comprising a vesicle derived from T lymphocyte and a HCV envelope glycoprotein or a CD81-binding fragment thereof;

---

3. claims: 1-9, 12, 22-28, 30 (all partially) and 17 (completely)

a method of producing lipid vesicles comprising: a) culturing a biological preparation comprising T lymphocytes, and b) collecting or purifying vesicles produced in a) wherein said lymphocytes are functionalized to express a selected molecule by indirect loading, a pharmaceutical composition comprising an indirectly loaded T cell derived membrane vesicle and a pharmaceutically acceptable vehicle or excipient, a method of stimulating an immune response against an antigen comprising administering an effective amount of said composition, a method of delivering an antigenic molecule to an antigen-presenting cell involving said composition, a method of stimulating dendritic cells involving said composition, a method of delivering a molecule to a target cell involving said composition, a composition comprising an immunogenic T cell derived membrane vesicle indirectly loaded with an antigenic molecule;

---

4. claims: 1-9, 12, 22-28 (all partially) and 18 (completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a method of producing lipid vesicles comprising: a) culturing a biological preparation comprising T lymphocytes having a determined T cell receptor, and b) collecting or purifying vesicles produced in a) wherein said lymphocytes are functionalized to express said specific T cell receptor at their surface, a pharmaceutical composition comprising a T cell derived membrane vesicle expessing a determined T cell receptor at their surface and a pharmaceutically acceptable vehicle or excipient, a method of stimulating an immune response against an antigen comprising administering an effective amount of said composition, a method of delivering an antigenic molecule to an antigen-presenting cell involving said composition, a method of stimulating dendritic cells involving said composition, a method of delivering a molecule to a target cell involving said composition,

---

5. claim: 19 (completely)

a method of producing vesicles comprising: a) culturing a T cell line, and b) collecting or purifying vesicles produced in a),

---

6. claim: 20 (completely)

a method of producing lipid vesicles comprising: a) culturing a biological preparation comprising T lymphocytes in the presence of a T cell activating agent, and b) collecting or purifying vesicles produced in a),

---

7. claim: 21 (completely)

a method of producing a pharmaceutical composition comprising: a) culturing a biological preparation comprising T lymphocytes, b) collecting of purifying vesicles produced in a), and c) conditioning said vesicles in a pharmaceutically acceptable carrier,

---

8. claim: 29 (completely)

a method of characterizing a preparation of vesicles derived from T cells.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int nal Application No
PCT/IB 03/01391

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0182958 A	08-11-2001	AU 6587301 A	12-11-2001
		CA 2407225 A1	08-11-2001
		CN 1426461 T	25-06-2003
		WO 0182958 A2	08-11-2001
		EP 1278825 A2	29-01-2003
		US 2001035132 A1	01-11-2001